Group B streptococcus colonization of Greek pregnant women and neonates: prevalence, risk factors and serotypes

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Objective To evaluate the prevalence and risk factors of group B streptococcus (GBS) colonization among pregnant women and their neonates in Greece, and to examine the serotype distribution of the GBS strains isolated and their susceptibility to antibiotics.

Methods A vaginal and a rectal swab were obtained from 1014 pregnant or parturient women followed at public and private hospitals in Athens and in a city of northern Greece. Cultures were also taken 24 h after birth from 428 neonates born to these women.

Results The overall maternal and neonatal colonization rates were 6.6% and 2.4%, respectively. The vertical transmission rate was 22.5%. By logistic regression analysis, multiparity (≥III) was associated with a lower colonization rate (odds ratio 4.4, 95% confidence interval 1.08–18.63). In contrast with other studies, middle-class women followed privately were more frequently colonized (10%) than those followed at the public hospital (3.9%) (odds ratio 3.1, 95% confidence interval 1.83–5.42). A higher number of prenatal visits was also associated with a higher colonization rate (change in true odds ratio when visits increased by one, 1.3; 95% confidence interval 1.14–1.60). No association was found between colonization and maternal age, previous obstetric history, marital status, nationality, prematurity, Caesarean section, or infant birth weight. The most common serotypes were II (26.9%), III (22.4%), Ia (19%), Ib (12%), and V (9%). A considerable proportion of the isolated strains was resistant to erythromycin (4.5%), clindamycin (6%), or both (6%).

Conclusion The rate and risk factors of maternal and neonatal GBS colonization may vary in different communities. These rates, as well as the incidence of neonatal disease, need to be thoroughly evaluated in each country to allow the most appropriate preventive strategy to be selected.

Keywords Group B streptococcus, colonization, maternal, neonatal, risk factors, serotypes, resistance

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INTRODUCTION

The group B streptococcus (GBS) has been identified as the leading cause of neonatal sepsis and meningitis in many European countries and in North America, causing considerable morbidity and mortality [1]. The incidence of these infections has been estimated to be about 0.5 to three cases per 1000 live births, and substantial geographic and racial differences have been identified [2,3]. Administration of intrapartum antimicrobial prophylaxis to colonized women has been shown to be effective in reducing the incidence of early-onset GBS disease in neonates [3]. Preventive strategies were therefore developed and have been introduced in North America and Australia,

where these methods have been shown to be cost-effective, given the rates of GBS disease in these countries [4]. However, studies from other parts of the world suggest that GBS is a less important cause of neonatal sepsis, and Gram-negative organisms are isolated more frequently [5]. Maternal colonization rates have also been found to vary between different countries or different ethnic groups within the same country, and appear to correlate with invasive infection rates in neonates [2,6].

The rate of GBS colonization and disease among pregnant women and their infants has not been studied in Greece, and no preventive strategy has been formulated with regard to intrapartum antimicrobial prophylaxis. The aim of this prospective, cross-sectional study was to examine the prevalence and risk factors of maternal and infant colonization in our area. In addition, the serogroup distribution of GBS strains and their susceptibility to antibiotics were also examined.

MATERIALS AND METHODS

One thousand and fourteen women were examined over a 17-month period between 1 January 2000 and 31 May 2001. Of all the participants, 589 were in labor, and the rest were enrolled during a follow-up examination at $\geq$35 weeks of gestation. Most of the women ($n = 617$) were examined at the Alexandra Maternity Hospital, which is a large, public, inner city, tertiary-care center in Athens, and of these, 29 were cared for by a private physician. Some women who were followed or delivered at two private maternity hospitals in Athens ($n = 181$) and at two private hospitals in the city of Drama, located in northern Greece ($n = 216$), were also enrolled in the study. A lower vaginal and a rectal swab were obtained from each subject. Cultures were also taken 24 h after birth from the ear canal, throat and umbilicus of 428 neonates born to women who were in labor at the time of enrollment in the study.

A standard questionnaire was filled out for each subject participating in the study. Information was obtained regarding demographic data, hospital of examination, insurance, gestational age, previous obstetric history, parity, marital status, number of prenatal visits, mode of delivery, administration of antibiotics during labor, neonate's birth weight, and the occurrence of sepsis. Oral informed consent was obtained from each patient. The study was approved by the Ethics Committee of the Alexandra hospital.

Microbiology

Swabs were immediately placed in transport medium (Transwab, Medical Wire and Equipment Co., Corsham, UK) and were inoculated within 48 h in Todd–Hewitt broth (Difco, Detroit, MI, USA) containing nalidixic acid (15 mg/L) and gentamicin (8 mg/L). After 24 h of incubation at 37 °C, subcultures were performed in Columbia 5% sheep blood agar (Sanofi Pasteur, Marmes La Coquette, France). Colonies were identified presumptively by colony morphology, Gram stain, and biochemical methods (Api 20 Strep, bio-Merieux, Marcyl' Etoile, France). Latex agglutination with the Streptococcus grouping kit (Oxoid, Basingstoke, UK) was used for serogroup determination. Susceptibility to antibiotics (penicillin, ceftriaxone, erythromycin, clindamycin, vancomycin, and chloramphenicol) was determined with a disk diffusion method, using Mueller–Hinton agar with 5% sheep blood. Susceptibility to penicillin, erythromycin and clindamycin was also examined with the Etest (AB Biodisk, Solna, Sweden), to determine the MIC. The results of both methods were interpreted according to the NCCLS recommendations [7]. Isolates were considered to be susceptible at the following MICs (mg/L): penicillin, $\leq$0.12; erythromycin, $\leq$0.25; and clindamycin, $\leq$0.25. Isolates were considered to be resistant to erythromycin and clindamycin at an MIC $\geq$1 mg/L. Strains were frozen at -20 °C until serotyping was performed with a coagglutination method, with antisera against types I–VIII provided by the Statens Seruminstitute, Copenhagen, Denmark.

Statistics

Subjects were divided into two groups based on the culture results. Women who were GBS carriers were compared to those with negative cultures according to their demographic, socio-economic and clinical characteristics. All 2×2 tables were analyzed using the chi-squared test or Fisher's exact test. Continuous variables were compared between subjects using one-way analysis of variance (one-way ANOVA).

A linear logistic model was fitted to evaluate the effects of all significant exposure factors observed. The dependent variable was GBS carriage, while the independent variables were: age, previous miscarriages, multiparity, marital status, hospital, number of prenatal visits, nationality, and insurance.

The level of significance was fixed at $\alpha = 5\%$. The statistical analysis was carried out with the software product SAS (SAS Institute, Cary, NC, USA), version 8.1.

RESULTS

Demographics

The mean age (± SD) of the subjects examined was 26.5 ± 5.3 years (median 27 years, range 15–46 years). The mean gestational age (± SD) at examination was 38.8 ± 1.57 weeks (median 39 weeks, range 31–42 weeks). Of the 1014 women, 812 (80%) were Greek, 136 (13.4%) Albanian, and 17 (1.7%) Gypsies; 49 (4.8%) belonged to other ethnic groups. Of all the women enrolled, 177 (13.5%) belonged to the lowest socio-economic class; they were followed at the public hospital, and did not have any insurance.

Colonization rate and risk factors

GBS were isolated from 67 women, and the overall colonization rate was 6.6%. The microorganism was cultured from vaginal swabs in 39 of 67 (58.2%) cases, from rectal swabs in seven cases (10.5%), and from both in 21 cases (31.3%). The characteristics of positive and negative women are shown in Table 1. There were no significant differences between colonized and GBS-negative women with regard to age, marital status, previous miscarriages or abortions, gestational age at

Table 1 Characteristics of pregnant women and their neonates

Characteristic	Women with negative cultures	GBS carriers	*P*-value	Odds ratio (95% CI)	Adjusted[a] odds ratio (95% CI)
Mean maternal age (years) ± SD	26.5 ± 5.4	26 ± 4.3	0.536		
Mother's age < 20	14.8%	12%	0.514	1.3 (0.60–2.75)	
Mean infant's birth weight (g) ± SD	3374 ± 402	3337 ± 303	0.529		
Previous miscarriages	8.9%	11%	0.503	1.3 (0.56–2.86)	
Multiparity (≥III)	14.3%	3%	0.005	5.4 (1.31–2.46)	4.5 (1.07–18.63)
Marital status					
Married	95.2%	98.5%	0.358	0.3 (0.04–2.23)	
Gestational age at delivery (weeks)±SD	39 ± 1.5	38.7 ± 1.4	0.069		
Prematurity at delivery (<37 weeks)	8%	9.4%	0.606	0.8 (0.31–2.20)	
Caesarean section	14.3%	16.3%	0.693	1.2 (0.53–2.59)	
Followed at the public hospital, no private physician ($n = 588$)	59.7%	34.3%	<0.001	2.8 (1.68–4.76)	3.1 (1.82–5.41)
Followed privately ($n = 426$)	40.3%	65.7%			
Mean number of prenatal visits ± SD	5 ± 2	5.8 ± 1.4	0.002		1.35[b] (1.13–1.60)
No insurance	14.1%	4.5%	0.025	3.5 (1.08–11.33)	
Nationality[c]					
Greek ($n = 812$)	79.3%	91%	0.017	2.7 (1.12–6.21)	
Albanian ($n = 136$)	13.4%	5.9%	0.065		
Gypsy ($n = 17$)	1.7%	1.5%	1.0		
Other ($n = 49$)	5%	1.5%	0.248		
Infant colonization	0%	16.7%	<0.001		

[a]After multivariate analysis.
[b]Estimated change in true odds if the number of visits increased by one.
[c]Ethnicity and insurance were not found to be significant in the multivariate analysis.
CI, confidence interval.

examination or at delivery, rate of prematurity, infant's birth weight, or route of delivery. Colonization rates in the different ethnic groups were: Greek 7.5%, Albanian 2.9%, Gypsies 5.8%, and other 2.1%. The colonization rate was significantly higher among Greek women compared to others (3%) ($P = 0.017$) (Table 1). Multiparous women (≥III) were less likely to be colonized (2/138, 1.4% versus 65/876, 7.4%) ($P = 0.005$). Women followed privately in Athens had a higher colonization rate (28/210, 13.3%) compared to those followed privately in northern Greece (16/216, 7.4%) and to women followed at the public hospital without a private physician, who had the lowest rate (23/588, 3.9%) ($P < 0.001$). A significant association was also found between colonization and number of prenatal visits. The mean (± SD) number of prenatal visits of the women with negative cultures was 5 ± 2, and that of those who were colonized was 5.8 ± 1.8 ($P = 0.002$). Women who belonged to the lowest socio-economic class and had no insurance were less frequently colonized (3/137, 2.2%) compared to the rest (64/877, 7.3%) ($P = 0.025$).

All significant exposure factors observed were evaluated by multivariate analysis. Of these, parity, hospital of examination or labor and number of prenatal visits were found to be statistically significant, whereas ethnicity and insurance were not (Table 1).

Neonatal colonization and disease

In total, 380 neonates born to GBS-negative mothers were examined, and none was colonized. Forty-eight neonates born to carrier mothers were examined, and nine were colonized. Thus, the overall infant colonization rate was 2.1%, and the rate of vertical transmission was 18.7%. GBS were isolated from the ear canal in seven of nine babies, from the throat in six of nine, and from the umbilicus in four of nine. Antibiotics were given at delivery to 51 of 380 (14.5%) GBS-negative mothers, and to 10 of 48 (20.8%) carriers whose neonates were examined ($P = 0.166$), and all these babies had negative cultures. The most common reason for administration of intrapartum antibiotics was the performance of Caesarean section (39/61), followed by prolonged rupture of membranes (14/61), and prematurity (5/61). If babies whose mothers received antibiotics were excluded, then the overall rate of colonization was 2.4% (9/369), and that of vertical transmission was 22.5% (9/40).

One of the colonized neonates developed asymptomatic bacteremia with GBS without complications, and was treated. Blood cultures were examined in this infant at birth because of a history of prolonged rupture of membranes. Intrapartum antibiotics were not given to the mother.

Serotype distribution

The serotype distribution of 67 GBS strains was as follows, in descending order: I 31.4% (Ia 19.4%, Ib 12%), II 26.9%, III 22.4%, V 9%, IV 3%, VI 3%, VII 3%, and VIII 1.5% (Table 2). The nine colonized neonates carried the same serotype as their mothers.

Table 2 Serotype distribution and resistance to erythromycin and clindamycin of 67 GBS strains isolated from pregnant and parturient women

	Erythromycin		Clindamycin			
Serotypes (%)	**I**	**R**	**I**	**R**	**Erythromycin and clindamycin[a]**	**Total resistant (%)**
Ia $n = 13$ (19.4)	1	1	1	–	–	3/13 (23)
Ib $n = 8$ (12)	–	–	1	1	–	2/8 (25)
II $n = 18$ (26.9)	–	–	1	–	1	2/18 (11)
III $n = 15$ (22.4)	1	–	–	–	2	3/15 (20)
IV $n = 2$ (3)	–	–	–	–	–	0/2
V $n = 6$ (9)	–	–	–	–	1	1/6(16.7)
VI $n = 2$ (3)	–	–	–	–	–	0/2
VII $n = 2$ (3)	–	–	–	–	–	0/2
VIII $n = 1$ (1.5)	–	–	–	–	–	0/1
Total $n = 67$		3/67 (4.5)	4/67 (6)		4/67 (6)	11/67 (16.5)

[a]All strains with reduced susceptibility to both antibiotics were resistant.
I, intermediate; R, resistant.

Table 3 Susceptibility of 67 GBS strains isolated from pregnant and parturient women to penicillin, erythromycin and clindamycin

Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	Percentage		
				Susceptible	Intermediate	Resistant
Penicillin	0.064	0.094	0.023–0.125	100	0	0
Erythromycin	0.125	0.25	0.016–8	89.5	3	7.5
Clindamycin	0.125	0.5	0.016–4	88	4.5	7.5

Susceptibility to antibiotics

All strains were susceptible to penicillin, 4.5% (3/67) were resistant to erythromycin, 6% (4/67) were resistant to clindamycin, and 6% (4/67) were resistant to both (Table 2). All strains were susceptible to chloramphenicol and vancomycin. The MIC results are shown in Table 3. The rates of resistance in different serotypes are shown in Table 2. There was no significant difference in the rate of resistance between the different serotypes ($P = 0.894$).

DISCUSSION

Many pregnant and parturient women from different geographic areas who belonged to different socio-economic classes and ethnic groups were examined for GBS colonization in this study. It was estimated that, overall, about 7% of the women and 2% of their infants were colonized with this microorganism. The maternal colonization rate was highest among middle-class pregnant women followed privately in Athens (13.3%), and lowest in those who were followed at the public hospital and did not have a private physician (3.9%). In a previous study conducted in Greece, the vaginal colonization rate among middle-class non-pregnant women with vaginitis was 10%, but selective broth was not used [8]. In another study of a small number of pregnant women from south-western Greece, the vaginal colonization rate was 12% [9]. Higher rates have been reported from other industrialized countries such as the USA and Canada, where estimates of GBS colonization rates among pregnant women are 15–40% [10,11]. In European countries, rates between 7% and 28% have been observed [12–17]. In the developing world, low rates of maternal colonization have been reported from some countries, and higher rates from others [6,18,19]. The overall colonization rate was low in this study, despite the examination of multiple sites and the use of adequate microbiological techniques. It is possible that genetic, social and cultural factors may play a role in maternal GBS colonization in a community.

The overall rate of vertical transmission was 19%, and that from infected mothers who did not receive intrapartum antibiotics to their infants was 22.5%. Although transmission rates range from 29% to 85% in most studies, rates as low as 12% have been reported from countries where low colonization rates have also been found despite the use of adequate microbiological methods [2,18].

Maternal colonization rates have been associated with infection rates in newborns. Although the data are very limited, it appears that the incidence of neonatal GBS disease is lower in Greece compared to that reported from several developed countries, where rates between 0.5 and 3/1000 births have been noted. From 1 January 1999 to 31 December 2001, about 13 500 births took place at the Alexandra public maternity hospital, and only two cases of invasive GBS disease were noted, accounting for a rate of 0.15/1000 births (unpublished data). One of these two neonates was asymptomatic, but GBS was isolated from blood cultures drawn at birth because of prolonged rupture of membranes. During the three-year period between 1 January 1997 and 31 December 1999, ten cases of early GBS disease were recorded among 37 823 births that took place at a large private maternity hospital in Athens, accounting for an incidence of 0.26/1000 births (D. Konstantinou, personal communication). However, the incidence of neonatal disease needs to be evaluated prospectively in a large birth cohort by a separate study. At the same time, current obstetric practices of intrapartum antibiotic use need to be recorded and evaluated.

Of the several risk factors examined, multiparity ($\geq$2 previous births) was associated with a low risk

for maternal colonization, in accordance with most previous studies [2,20]. The place of examination and the number of prenatal visits were also associated with GBS colonization. The results of multivariate analysis showed that women who were followed privately and those with a higher number of prenatal visits were more frequently colonized. These women belong to a higher socioeconomic class compared to those who seek care at a public inner city hospital and have poor prenatal care. This finding is in contrast with similar studies conducted in different settings in which GBS colonization has been found more frequently among women of low socio-economic class followed at public hospitals [2,20].

Serotype distribution was almost even between I, II and III, with a slight preponderance of serotype I. Serotype V, which has recently emerged as a frequently colonizing serotype in several countries [21], accounted for a considerable proportion of the strains (9%), ranking fourth. With the exception of serotype V, which, in our study, was half as common, the serotype distribution found was not much different from the one detected in a recent study from the USA, where higher colonization rates were observed [11]. Therefore, serotype distribution may not account for the lower colonization rate observed in our area.

All strains examined were susceptible to penicillin, but a considerable proportion was resistant to erythromycin (10.5%) and clindamycin (12%). The rate of erythromycin resistance compares well with that found in a study conducted during 1991 among GBS colonizing strains isolated in Athens, where it was 9% [22]. Increasing rates of GBS resistance have been recently reported from developed and developing countries [23–26]. These findings support the use of penicillin for the prevention and treatment of these infections, and raise concern about the use of erythromycin and clindamycin in penicillin-allergic patients.

In conclusion, maternal and neonatal GBS colonization rates were found to be lower in this study than in those reported from other developed and from some developing countries. The risk factors associated with maternal colonization were also different. These findings may be related to genetic, cultural or social factors, and indicate that the profile of GBS colonization and infection may vary in different communities. An accurate evaluation of the colonization rate and, more importantly, the neonatal invasive disease incidence is required for the selection of the appropriate preventive strategy in each country [27].

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